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19. KEY WORDS (Continue on reverse side if necessary and identify by block number) cofactor electrodes, immobilized enzymes, flavin, FAD		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This work was focused on the study of flavin cofactor enzymes for the development of improved oxidation-reduction enzyme electrodes for eventual use in biosensors or other electrochemical systems. Flavin adenine dinucleotide or glucose oxidase were immobilized on different types of carbon electrodes and tested for electrochemical and enzymatic activity. Diffusional studies also were carried out to determine the diffusional resistance of the immobilization matrix.		

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ENZYME COFACTOR MODIFIED ELECTRODES

Final Report

Lemuel B. Wingard Jr.

19 February, 1986

U. S. Army Research Office

Contract DAAG29-82-K-0064

University of Pittsburgh

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#### A. Problem Studied

This contract work has been part of a longer range study aimed at the development of greatly improved oxidation-reduction enzyme electrodes. Such electrodes should have very significant applications in analytical chemistry (i.e. in field use biosensors or in clinical laboratory analyses) and in other areas where oxidation-reduction chemistry is important. This contract work has been focused on flavin enzymes since the flavin cofactor system is present in a large number of oxidation-reduction enzymes and since the flavin cofactor presents some possibilities for novel cofactor-electrode interactions not compatible with non-flavin type cofactors. The key thrust of this contract work has been to attach the flavin cofactor, rather than the apoprotein portion of the enzyme, to a solid electrode surface and to examine how the point or method of attachment influences 1) the electrochemical activity of the attached flavin, 2) the enzymatic activity of the attached flavin with added apoenzyme, and 3) the rates of diffusion of substrates or mediators through the immobilization matrix.

#### B. Major Accomplishments

The flavin cofactor for glucose oxidase, flavin adenine dinucleotide (FAD), was attached by adsorption to spectroscopic graphite. The adsorbed material was electrochemically active (by cyclic and differential pulse voltammetry measurements) and showed partial reconstitution of enzyme activity upon addition of apoglucose oxidase. The mechanism of this reconstitution needs to be explored further since the FAD appears to remain adsorbed on the carbon surface. FAD was attached by covalent means, through 1) the adenine amino group on FAD and 2) most probably the ribityl OH group on FAD, to glassy carbon electrodes. Attachment at either of these two functional groups led to electrochemical activity but not enzymatic activity. These results were in keeping with the concept that both the ribityl OH and adenine amino parts of FAD are buried deep within the apoenzyme molecule and thus are not accessible to the surface of the enzyme. Attachment at position 8 of the isoalloxazine ring system, however, has been shown to give electrochemical as well as partial enzymatic activity so that the concept of covalent attachment of FAD to the electrode surface definitely merits further work (Note: the attachment at position 8 was done under NSF sponsorship and is mentioned here only for comparison purposes). Recent work under this ARO contract, whereby holo glucose oxidase (FAD complexed with apoenzyme) was attached covalently to aminophenylboronic acid activated glassy carbon, gave enhanced electron transfer as well as partial enzymatic activity. One can surmise that the aminophenylboronic acid immobilization occurred via complexation with the glucose oxidase carbohydrate portion and that this led to a conformational change that better exposed the FAD molecule to the electrode surface. The enhanced electron transfer was an unexpected result that needs to be explored further as to mechanism and useful applications. Measurement of diffusional resistances, brought about by the immobilization matrix, was undertaken using rotating disc or rotating ring-disc electrodes; and a method was developed for the simultaneous determination of the diffusion coefficient and partition coefficient of analytes within the immobilization matrix.

Methodologies also were explored for the measurement of electron transfer rate constants and for infrared determination of the structural chemistry of the immobilized flavins.

The study of flavin electrodes continues to hold considerable promise for the development of more efficient oxidation-reduction enzyme electrodes. This ARO contract work has defined several immobilization schemes and experimental methodologies that will be very useful in obtaining an in-depth knowledge of how spatial orientation and electron transfer mechanisms can relate to the development of improved enzyme electrodes. A renewal contract application is forthcoming.

### C. List of Publications

#### 1. Papers

- (a). Wingard Jr., L. B. (1983), Prospects for Electrochemical Devices and Processes Based on Biotechnology, Proc. Biotech. '83 Online Publications Inc., Northwood, U.K., pp. 613-624.
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- (k). Wingard Jr., L.B. (1985) Redox Enzyme Cofactor Systems in Biotechnology, in Proc. 16th FEBS Congress, (Part C), VNU Science Press, Utrecht, pp. 187-190.
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- (m). Wingard, Jr., L.B. and Narasimhan, K. ( ) Electron Transfer with a Glassy Carbon/Phenylboronic Acid/Glucose Oxidase Electrode, Proc. 1985 Conf. on Chem. Res. Def., Aberdeen Prov. Gnd., MD, pp. (in press).
- (n). Wingard, Jr., L.B. and Narasimhan, K. ( ) Immobilized Flavin Coenzyme Electrodes for Analytical Applications, in Immobilized Enzymes and Cells, K. Mosbach, ed. (Meth. Enzymol. vol. ), Academic Press, New York (in press).
- (c). Narasimhan, K. and Wingard Jr., L.B. ( ) Direct Electron Transfer with Glucose Oxidase Immobilized on Aminophenylboronic Acid Modified Glassy Carbon, Ann. N.Y. Acad. Sci. (in press).
- (p). Narasimhan, K. and Wingard Jr., L.B. ( ) Enhanced Direct Electron Transport with Glucose Oxidase Immobilized on Aminophenylboronic Acid Modified Glass Carbon Electrodes, Anal. Chem. (submitted).
- (q). Marrese, C.A., Miyawaki, O., and Wingard Jr., L.B. ( ) Simultaneous Electrochemical Determination of Diffusion and Partition Coefficients of Potassium Ferrocyanide for Albumin-Glutaraldehyde Membranes (in preparation).
- (r). Narasimhan, K. and Wingard Jr., L.B. ( ) FAD Immobilized on Aminophenylboronic Acid (in preparation).

## 2. Abstracts

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- (b). Wingard Jr., L.B., Castner, J.F., Miyawaki, O. and Marrese, C. (1983) Rotating Ring-Disc Electrode: Its Use in Studying Mass Transport Resistances through Immobilized Enzyme Support Matrices, Abst. 7th Intl. Enzyme Eng. Conf., White Haven, PA, Sept. 25-30. p. 53.
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#### D. Participating Scientific Personnel

The following professional level scientific personnel participated in this project:

1. Lemuel B. Wingard Jr., Ph.D.
2. Osato Miyawaki, Ph.D.
3. Carl A. Marrese, Ph.D.
4. Krishna Narasimhan, Ph.D.

No degrees were awarded to participants of this work.